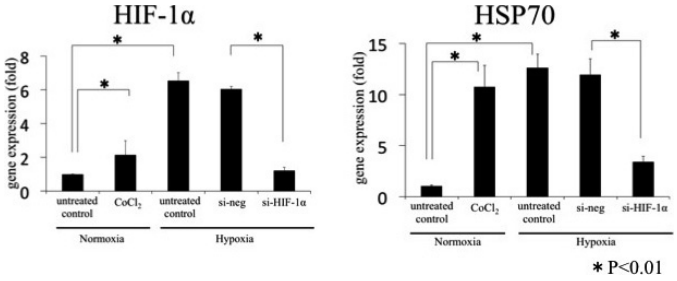
Results: The cells without siRNA or CoCl2 treatment were designated as the untreated control cells. The untreated control cells under hypoxia revealed a significant increase in the HIF-1α mRNA levels compared with cells cultured under normoxia. HIF-1α mRNA expression was significantly increased in chondrocytes cultured under simulated hypoxia in comparison with the untreated cells under normoxia. Under condition of hypoxia, si-HIF-1α reduced HIF-1α mRNA levels to about 15.2% at 24hours after transfection, compared to non-targeting scramble siRNA (si-neg) treated cells. The mRNA levels of HSP70, PG, and ColII under hypoxia and simulated hypoxia were significantly increased in comparison with chondrocytes cultured under normoxia. Under condition of hypoxia, the mRNA levels of HSP70, PG, and ColII, were significantly suppressed by si-HIF-1α transfection.
Conclusions: Up-regulation of HSP70 under hypoxia is part of the low oxygen stress response seen in Drosophila, and mammalian tissues. Up-regulation of heat shock factor (HSF), by which HSP70 are known to be regulated, under hypoxia requires the activity of HIF-1α, the effector of the low oxygen response. In this study, HIF-1α and HSP70 mRNA expressions in cultured chondrocytes were significantly increased under condition of hypoxia and simulated hypoxia. These expressions were not increased in chondrocytes by transfection with si-HIF-1α. These results suggest that HSP70 might be transcriptionally regulated by HSF through HIF-1α in cultured chondrocytes under hypoxia. We previously reported that the expression of HSP70 had an important role for PG and ColII synthesis with heat stimulation in rabbit articular cartilage. In this study, the expression of HSP70, PG, and ColII in cultured chondrocytes were significantly increased under condition of hypoxia, and were increased with CoCl2 under normoxia. But these expressions were not increased under hypoxia in which HIF-1α was inactivated by si-HIF-1α transfection. These findings suggest that PG and ColII gene might be regulated via the HIF-1α and HSP70 pathway under hypoxia.



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DICKKOPF-3 CAN REGULATE CARTILAGE DEGRADATION AND CHONDROCYTE CELL SIGNALLING

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Purpose: To investigate the role of Dickkopf-3 (Dkk3) in Osteoarthritis (OA).
We have previously shown that Dkk3 expression is increased in OA cartilage and synovium. Additionally levels of Dkk3 in synovial fluid are in individuals with tricompartmental OA after arthroscopy. The factors regulating Dkk3 expression in cartilage and the effect of Dkk3 on chondrocyte function are poorly ascribed.
Correct regulation of cell signalling pathways is integral to cartilage homeostasis and thus the prevention of OA pathogenesis. Dkk3 is a member of the Dkk family of Wnt antagonists and therefore may impact on chondrocyte biology through interaction with the Wnt pathway. Dkk3 has also been found to influence TGFbeta signalling in other cell systems
Methods: Expression of Dkk3 was assessed in primary human articular chondrocytes (HACs) following treatment with interleukin-1 (IL1) and oncostatin-M (OSM). The effect of Dkk3 on IL1/OSM-induced proteoglycan and collagen release from bovine nasal cartilage explants and primary human chondral explants was assessed using the DMMB and hydroxyproline assays. SW1353 chondrosarcoma cells were treated with Dkk3 +/− Wnt3a or TGFbeta and TOPFlash and CAGA luciferase reporters used to measure Wnt and TGFbeta signalling respectively. RNA was extracted from primary HACs treated with Dkk3 +/− TGFbeta or Wnt3a. ADAM12 and TIMP3 expression were measured to assess TGFbeta signalling and AXIN2 expression measured to assess Wnt signalling.
Results: Dkk3 expression was decreased in primary HAC following IL1/OSM treatment. In bovine nasal cartilage explants, IL1/OSM-induced proteoglycan release was inhibited by Dkk3. Dkk3 antagonized canonical Wnt signalling, decreasing Wnt3a-induced AXIN2 expression and Wnt3a-induced luciferase expression from the TOPFlash reporter. Interestingly, Dkk3 appears to enhance TGFbeta signalling, increasing TGFbeta-induced TIMP3 and ADAM12 expression and TGFbeta-induced luciferase from the CAGA-luc reporter
Conclusions: OA pathogenesis is likely regulated by a multitude of factors relating to cell signalling including the balance of cytokines present in the articular joint. Dkk3 expression is increased in OA but we have found that it can still be regulated by OA-relevant cytokines IL1 and OSM. This suggests a balance of Dkk3 effects depending upon the biological stimuli within the cartilage. Dkk3 may act in a protective role in the presence of inflammatory cytokines as exemplified by its ability to inhibit matrix loss from chondral explants. Furthermore the ability of Dkk3 to antagonize Wnt signalling and enhance TGFbeta signalling implies that Dkk3 could influence multiple OA-relevant processes and be important in cartilage development and homeostasis.

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CYTOKINE INDUCED MULTIMERIZATION OF SYNDECAN-4 IS MEDIATED BY GAG SIDE CHAINS

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Purpose: Syndecan-4 (SDC4) is a transmembrane heparan sulphate proteoglycan, which can act as a membrane receptor constituting binding sites for an array of ligands, such as TGF beta. Although it has been shown that SDC4 binds a variety of extracellular ligands, the exact mechanisms concerning SDC4 signaling are still unknown. As we could show in our previous study, the antibody-mediated blockade of SDC4 function as well as its complete loss leads to protection of osteoarthritis induction and cartilage degradation in mice. Thus, we investigated the underlying mechanisms leading to reduced cartilage destruction in osteoarthritis.
Methods: In order to elucidate SDC4 signaling, different side chain mutants were designed using overlap PCR. We mutated the serine residues, which constitute the heparan sulphate attachment sites to alanine, to abolish side chain attachment. Laser scanning fluorescence microscopy of transient transfected Cos-7 cells was performed to ensure membrane localization of generated mutants. The multimerization pattern of different mutants was analysed using crosslinking upon cytokine stimulation and subsequent western blot analysis.
Results: All side chain lacking SDC4 mutants exhibited normal intracellular trafficking into the cell membrane. While wildtype SDC4 shows normal multimerization, the side chain mutants exhibited an impaired tetramer formation. We showed that SDC4 is known to be expressed as a monomere and shows dimer formation upon cytokine stimulation. Interestingly, the side chain lacking mutants were less able to form dimers than the wildtype.
Conclusions: We could show that side chains are essential for multimerization. Moreover, our results demonstrate that cytokine stimulation promotes the multimerization process. Therefore, SDC4 multimerization might be an important step in signal transduction during osteoarthritic cartilage damage.

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FIBRONECTIN FRAGMENT ACTIVATES AKT LEADING TO NF-κB UP-REGULATION IN OSTEOARTHRITIC CHONDROCYTES

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Background: Increased fibronectin fragments are thought to contribute to joint destruction in osteoarthritis (OA). However, the mechanism whereby fibronectin fragments cause catabolic activities is not totally understood. While COOH-terminal heparin-binding fibronectin fragment (HBFN-f) has been shown to activate nuclear factor (NF)-κB pathway, intracellular upstream events that cause NF-κB up-regulation in response to HBFN-f remain unclear.
Purpose: This study was aimed to elucidate the involvement of phosphoinositide-3-OH kinase (PI3K)/Akt pathway in NF-κB activation by HBFN-f in OA chondrocytes.
Methods: Chondrocytes isolated from articular cartilage from OA knee joints were cultured in monolayer with HBFN-f. Secreted levels of nitric